Highly parallelized droplet cultivation and prioritization on antibiotic producers from natural microbial communities

Lisa Mahlera,b, Sarah P. Niehsc, Karin Martina, Thomas Webera, Kirstin Scherlachc, Christian Hertweckc,b, Martin Rotha, Miriam A. Rosenbauma,b\*

aBio Pilot Plant, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, 07745 Jena, Germany;

bFaculty of Biological Sciences, Friedrich Schiller University, 07743 Jena, Germany

cBiomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, 07745 Jena, Germany

\*Correspondence: miriam.rosenbaum@leibniz-hki.de (M.A.R.)

SUPPLEMENTARY INFORMATION

**List of tables**

|  |  |  |
| --- | --- | --- |
| 1 | Screenings for antibiotics in droplets with 2 different reporter strains and 2 different media… | 2 |
| 2 | Diameter of inhibition zones for selected isolate D121-0906-b3-2-1………………………………………. | 2 |
| 3 | Media compositions for droplet cultivation of soil community……………………………………………….. | 2 |
| 4 | Media compositions for reporter strain cultivation…………………………………………………………………. | 3 |
| 5 | Media compositions for cultivation of isolates for antimicrobial activity testing……………………… | 4 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Medium | Reporter strain | Nb. ofisolates | Nb. of isolates characterized |
| 1 | 50% CESE, 6% soy mannit medium | *E. coli* | 67 | 17 |
| 2 | 50% CESE, 6% malt medium | *E. coli* | 132 | 68 |
| 3 | 50% CESE, 6% soy mannit medium | *B. subtilis* | 180 | 63 |
| 4 | 50% CESE, 6% malt medium | *B. subtilis* | 78 | 9 |

**Table S 1** – Screenings for antibiotics in droplets with 2 different reporter strains and 2 different media.

**Table S 2** – Diameter of inhibition zones for selected isolate D121-0906-b3-2-1.

 The antibiotics \*1Ciprofloxacin 5 g/mL and \*2Amphothericin B 10 g/mL were used as positive controls.

|  |  |  |
| --- | --- | --- |
| Test strain | 0906-b3-2-1 | Control |
| *Bacillus subtilis* | 15 | 28\*1 |
| *Staphylococcus aureus* | 18/23 | 18\*1 |
| *Escherichia coli* | 18 | 23/30\*1 |
| *Pseudomonas aeruginosa* | 17 | 27/33\*1 |
| *Mycobacterium vaccae* | 15 | 21\*1 |
| *Sporobolomyces salmonicolor* | 13/20 | 18\*2 |
| *Candida albicans* | 45/48 | 21\*2 |
| *Penicillium notatum* | 45 | 19\*2 |

**Table S 3** – Media compositions for droplet cultivation of soil community.

|  |  |
| --- | --- |
| Medium | Composition |
| SM | soy mannitol medium; 20 g/L soy coarse meal (Schkade Landhandel, Germany) + 20 g/L mannitol (Merck, Germany) in distilled water, pH adjusted to 6.5, 35 min at 121 °C |
| 0.06SM 0.5CESE | 6% (v/v) supernatant of soy mannitol medium (see above) + 50% (v/v) cold extracted soil extract + 44% (v/v) distilled water |

|  |  |
| --- | --- |
| Medium | Composition |
| TB + 1% glucose | 12 g/L tryptone (Bacto Tryptone, BD Bioscience, Belgium) + 24 g/L yeast extract (Bacto Yeast Extract, BD Bioscience, Belgium) + 4 g/L glycerol (Roth, Germany) in tap water, pH adjusted to 7.2 with NaOH, 20 min 121 °C, + 0.17 M KH2PO4 (Merck, Germany) + 0.72 M K2HPO4 (Merck, Germany) + 1% (w/v) glucose (VWR International, USA) |
| 2.5x TB + 1% glu-cose | 30 g/L tryptone (Bacto Tryptone, BD Bioscience, Belgium) + 60 g/L yeast extract (Bacto Yeast Extract, BD Bioscience, Belgium) + 10 g/L glycerol (Roth, Germany) in tap water, pH adjusted to 7.2 with NaOH, 20 min 121 °C, + 0.425 M KH2PO4 (Merck, Germany) + 1.8 M K2HPO4 (Merck, Germany) + 1% (w/v) glucose (VWR International, USA) |

**Table S 4** – Media compositions for reporter strain cultivation.

|  |  |
| --- | --- |
| Medium | Composition |
| 0.3SM 0.2CESE | 30% (v/v) supernatant of soy mannitol medium (see above) + 20% (v/v) cold extracted soil extract + 50% (v/v) distilled water |
| 0.12SM 0.5CESE | 12% (v/v) supernatant of soy mannitol medium (see above) + 50% (v/v) cold extracted soil extract + 38% (v/v) distilled water |
| 0.5SM | 50% (v/v) supernatant of soy mannitol medium (see above) + 50% (v/v) distilled water |
| MMM | Modified Malt Medium; 2 g/L yeast extract (Bacto Yeast Extract, BD Bioscience, Belgium) + 2 g/L beef extract + 15 g/L malt extract in distilled water, pH adjusted with NaOH to 7.2, 20 min 121 °C |
| 0.5MMM 0.2CESE | 50% (v/v) modified malt medium (see above) + 20% (v/v) cold extracted soil extract + 30% (v/v) distilled water |
| NBE | 1 g/L beef extract + 2 g/L yeast extract (Bacto Yeast Extract, BD Bioscience, Belgium) + 5 g/L bact. peptone (Bacto Soytone, BD Bioscience, Belgium) + 5 g/L NaCl (Merck, Germany) in distilled water, 20 min 121 °C |
| NBE + 10Gluc | NBE (see above ) + 10 g/L glucose (VWR International, USA) |
| Soja2g | 15 g/L soy coarse meal (Schkade Landhandel, Germany) + 15 g/L glucose (VWR International, USA) + 5 g/L NaCl (Merck, Germany) + 1 g/L CaCO3 (Merck, Germany) + 0.3 g/L KH2PO4 (Merck, Germany) in distilled water, 20 min 121 °C |
| Soja2e | 20 g/L soy coarse meal (Schkade Landhandel, Germany) + 20 g/L glucose (VWR International, USA) + 5 g/L NaCl (Merck, Germany) + 1 g/L CaCO3 (Merck, Germany) in distilled water, 20 min 121 °C |
| M65 | 4 g/L yeast extract (Bacto Yeast Extract, BD Bioscience, Belgium) + 4 g/L glucose (VWR International, USA) + 10 g/L malt extract in distilled water, 20 min 121 °C |
| MGY M9 | 1 g/L (NH4)2SO4 (Merck, Germany) + 0.1 g/L MgSO4 (Roth, Germany) + 0.588 g/L Sodium Citrate (VWR International, USA) + 7 g/L KH2PO4 (Merck, Germany) + 2 g/L K2HPO4 (Merck, Germany) + 1.25 g/L yeast extract (Bacto Yeast Extract, BD Bioscience, Belgium) + 10 g/L glycerol (Roth, Germany) in distilled water, 20 min 121 °C |
| MGY + Gluc | MGY M9 (see above ) + 10 g/L glucose (VWR International, USA) |

 **Table S 5** – Media compositions for cultivation of isolates for antimicrobial activity testing.